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14. ABSTRACT We build on our earlier findings of three potential contributing genetic factors (ADRB2, TLR4, CFH)in the development of heterotopic ossification (HO). HO development in long bone fractures correlated with all of these variables but was inversely associated with head injury scores. With more recent data we have determined that the ADRB2 minor allele is associated with HO formation in our patient population however, TLR4 and CFH no longer demonstrate significance.					
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INTRODUCTION

The goals of our study have been to understand the possible genetic influences on the formation of heterotopic ossification (HO) after bony injury. One of our hypotheses is that the systemic response to the physiologic stress of trauma differs between individuals based on their genetic predisposition. Our working formula is: **Environment (physiologic stress) + Genetics = Outcome (mortality, HO, etc).**

We have examined our database of long bone and acetabulum/pelvic fractures (n=1313) for HO after fracture. Radiographs were reviewed by a musculoskeletal radiologist and a fellowship trained orthopedic traumatologist. Of the 1313 patients, 1128 survived and had >8weeks follow up. There were 125 patients with HO (11%) consistent with incidences in the literature for civilian trauma.

Previously, we demonstrated an association of three single nucleotide polymorphisms (SNPs) with risk for the development of heterotopic ossification (HO) after traumatic fracture. The CC polymorphism for the B2 adrenergic receptor was associated with an increased risk of forming HO. Polymorphisms for Toll-Like Receptor 4 and Complement Factor H on the other hand appeared to be protective. However, this was preliminary data based on a small subset of patients and HO defined in the medical record. We have now verified HO with radiographic examination and have found only one of these to be associated with HO. With our revised, and we believe more accurate, data the GG allele of ADRB2 is associated with an increased risk of HO (p=0.039). This differs from our prior results but still implicates this ADRB2 gene in Pathways that lead to HO formation. The previous finding of CFH and TLR4 alleles associated with a decreased likelihood of HO are no longer found to correlate in this new dataset.

There has been a significant delay in this past year due to multiple factors: 1. The PI (EJM) transferred to Loyola University Medical Center from Vanderbilt University in September 2010. Work on this project continued by way of remote access and monthly visits to Vanderbilt. 2. The geneticist (JC) working with our team took an early retirement secondary to health concerns. 3. Administrative changes in the Department of Trauma at Vanderbilt University has led to a change in the position of two members of the research team (JAM, PRN). 4. Inadvertent loss of remote access for EJM, now restored. 5. Updates in our core's technology have delayed gene extraction and genotyping. Fortunately, these updates ultimately have allowed us to greatly increase the number of genetic polymorphisms we will examine which will improve our understanding of the genetic underpinnings of HO formation. We are grateful to have received a no-cost extension for this work to be completed.

BODY

Aim 1: To examine the relationship of genetics to the Heterotopic Ossification phenotype

Extraction has been completed on 5128 patients. Genotyping will begin in the next month. We have generated an expanded list of polymorphisms to examine in this population. Previously, in our pilot data we demonstrated a relationship between polymorphisms of three genes, Complement Factor H, Toll-Like Receptor 4 and a B2 Adrenergic Receptor. Our most recent data only demonstrates a statistically significant relationship between HO and the GG allele of ADBR2 which is contrary to our previous result of the CC polymorphism being the predictor. However, this ADBR2 gene still appears related to HO which continues to support our hypothesis that autonomic dysregulation may contribute to the formation of HO.

Aim2: To determine the environmental effect (ie. injury severity, traumatic brain injury, medications) on phenotypic expression

We compiled data on 1313 patients including demographics, injury severity score (ISS), head abbreviated injury severity score (AIS head), ICU days, and ventilator days. Higher AIS head scores were found to be inversely associated with the development of HO. Several recent studies have demonstrated similar results which are contrary to popular previous data. This may be a result of more recent studies basing head trauma severity on the AIS head score instead of the Glasgow Coma Scale which is less indicative of actual head injury. The analysis was performed by a faculty level statistician in the Department of Biostatistics at Vanderbilt University.

Aim3: To determine clinical biomarkers which predict the HO phenotype

The data has been captured for the 6000 patients with specimens in the repository. Extraction has been performed on 5128 specimens thusfar. We will organize this data once we are certain which patients in the database have been successfully genotyped and meet the inclusion criteria.

KEY RESEARCH ACCOMPLISHMENTS

- DNA extraction performed on 5128 specimens
- Data has been compiled for 1313 patients including:
 - ISS
 - Head AIS
 - Number ICU days
 - Ventilator Days
 - Ventilator Assisted Pneumonia
 - Age
 - Race
 - Gender
- Publication: *The Genetics of Heterotopic Ossification: Insight into the Bone Remodeling Pathway* J Orthop Trauma. 2010 Sep;24(9):530-3.
- Presented at the OTA Annual Meeting, San Diego CA 2009
- Presented at the Extremity War Injuries Symposium, Washington DC 2010
- Accepted for poster presentation at OTA 2011

REPORTABLE OUTCOMES

Revised data using radiographic findings of HO

	No HO	HO	p-Value
Age	40.67 ± 17.89	42.69 ± 16.38	0.087
ISS	24.84 ± 12.08	28.35 ± 11.75	0.003
AIS Head	2.13 ± 1.84	1.70 ± 1.80	0.016
Hosp Days	12.81 ± 12.84	18.42 ± 17.28	<0.001
ICU Days	5.61 ± 7.32	8.14 ± 8.75	<0.001
Vent Days	4.66 ± 7.11	6.60 ± 6.91	<0.001

HO vs No HO for each polymorphism using revised data

Polymorphism	p - value	p- value Previous Result
ADBR2 GG	0.421	0.031
ADBR2 GC	0.153	0.054
ADBR2 CC	0.039	0.533
TLR4 CC	0.210	0.642
TLR4 CT	0.142	0.053
TLR4 TT	0.220	0.239
CFH CC	0.161	0.643
CFH CT	0.826	0.255
CFH TT	0.209	0.030

CONCLUSION

The ADBR2 gene that we examined (rs1042714) is once again shown to be associated with HO formation after fracture. This and recent literature continues to suggest a possible role of the systemic inflammatory response in contributing to HO formation.

The abbreviated injury severity score for head injury was not associated with an increased incidence of HO in this population. To the contrary, it was more likely for a patient with a lower AIS head score to have HO than those with higher head injury scores.

We have greatly increased our candidate polymorphism list with the advent of improved genotyping technology. We currently plan to examine 390 SNPs for 34 genes associated with multiple inflammatory, immunologic and bone remodeling pathways. The SNPs for each gene of interest were identified using Phase III of the International HapMap Project (www.HapMap.org) to identify independent regions of each gene to avoid inclusion of SNPs that overlap. Preference was given to SNPs that have been reported in the literature.

APPENDIX

Revised List of gene regions to be examined. Specific rs numbers are not listed but total 390 SNPs.

CFH
CRP
TLR4
ADRB1
ADRB2
ADRB3
ADRBK1
ADRA1A
ADRA1B
ADRA2
VEGFA
BMP2
BMP4
ACVR1
LRP5
VDR
TNFRSF11A
TNFSF11
IL1A
IL1B
IL6
IL10
TNF
GNAS
EXT1
EXT2
SOST
HLABC-CA
NF1
LEPTIN
HMGB1
WNT
PPAR
IBSP
SPP1
PAQR6